1: J Mol Biol 1999 Mar 26;287(2):331-46 Related Articles, Links

FULL-TEXT ARTICLE

# Protein mimicry of DNA from crystal structures of the uracil-DNA glycosylase inhibitor protein and its complex with Escherichia coli uracil-DNA glycosylase.

**Putnam CD, Shroyer MJ, Lundquist AJ, Mol CD, Arvai AS, Mosbaugh DW, Tainer JA.**

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Uracil-DNA glycosylase (UDG), which is a critical enzyme in DNA base-excision repair that recognizes and removes uracil from DNA, is specifically and irreversably inhibited by the thermostable uracil-DNA glycosylase inhibitor protein (Ugi). A paradox for the highly specific Ugi inhibition of UDG is how Ugi can successfully mimic DNA backbone interactions for UDG without resulting in significant cross-reactivity with numerous other enzymes that possess DNA backbone binding affinity. High-resolution X-ray crystal structures of Ugi both free and in complex with wild-type and the functionally defective His187Asp mutant Escherichia coli UDGs reveal the detailed molecular basis for duplex DNA backbone mimicry by Ugi. The overall shape and charge distribution of Ugi most closely resembles a midpoint in a trajectory between B-form DNA and the kinked DNA observed in UDG:DNA product complexes. Thus, Ugi targets the mechanism of uracil flipping by UDG and appears to be a transition-state mimic for UDG-flipping of uracil nucleotides from DNA. Essentially all the exquisite shape, electrostatic and hydrophobic complementarity for the high-affinity UDG-Ugi interaction is pre-existing, except for a key flip of the Ugi Gln19 carbonyl group and Glu20 side-chain, which is triggered by the formation of the complex. Conformational changes between unbound Ugi and Ugi complexed with UDG involve the beta-zipper structural motif, which we have named for the reversible pairing observed between intramolecular beta-strands. A similar beta-zipper is observed in the conversion between the open and closed forms of UDG. The combination of extremely high levels of pre-existing structural complementarity to DNA binding features specific to UDG with key local conformational changes in Ugi resolves the UDG-Ugi paradox and suggests a potentially general structural solution to the formation of very high affinity DNA enzyme-inhibitor complexes that avoid cross- reactivity. Copyright 1999

1: Mol Cell Biol 1984 Mar;4(3):407-14 Related Articles, Links

# Expression of plasmid R388-encoded type II dihydrofolate reductase as a dominant selective marker in Saccharomyces cerevisiae.

**Miyajima A, Miyajima I, Arai K, Arai N.**

The R388 plasmid-encoded drug-resistant type II dihydrofolate reductase gene (R . dhfr) was expressed in Saccharomyces cerevisiae by fusing the R . dhfr coding sequence to the yeast TRP5 promoter. Yeast cells harboring these recombinant plasmids grew in media with 10 micrograms of methotrexate per ml and 5 mg of sulfanilamide per ml, a condition which inhibits the growth of wild-type cells. Addition of a 390-base-pair fragment from the 3'-noncoding region of TRP5 downstream from R . dhfr increased expression. Presumably, the added segment promoted termination or polyadenylation or both of the R . dhfr transcript. The activity of the plasmid-encoded dihydrofolate reductase and the copy number of the R . dhfr plasmid in cells grown in drug-selective media were higher by one order of magnitude than those grown in nutrition-selective media. Plasmid copy number, as well as the plasmid-encoded enzyme level, decreased when cells were selected for prototrophy. In drug-selective media, the plasmid-encoded enzyme level and the content of R . dhfr transcripts were nearly constant in cells harboring R . dhfr plasmids containing different yeast promoters. In contrast, the plasmid copy number and beta-lactamase activity encoded in cis by plasmids were much higher when R . dhfr was associated with the weak TRP5 promoter than when it was fused to the strong ADC1 promoter. These results indicate that plasmid copy number, i.e., gene dosage of R . dhfr, correlates inversely with the strength of the promoter associated with R . dhfr, and cells with a higher plasmid copy number were enriched in drug-selective media.(ABSTRACT TRUNCATED AT 250 WORDS)

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1: Gene 1991 Mar 1;99(1):31-7 Related Articles, Links

# Overproduction and characterization of the uracil-DNA glycosylase inhibitor of bacteriophage PBS2.

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A plasmid expression vector (pZWtac1) was constructed which allowed inducible overexpression of the uracil-DNA glycosylase (Ung) inhibitor (Ugi)-encoding gene (ugi) in Escherichia coli. In this plasmid, the ugi gene was under the control of both its own promoter and the tac promoter. Constitutive expression of the ugi was observed in the absence of isopropyl-beta-D-thiogalactopyranoside (IPTG). In the presence of 1 mM IPTG, the Ugi protein was overproduced to an approx. 16-fold higher level, and accounted for approx. 19% of the total soluble cellular proteins. Following high-level production in E. coli, the Ugi protein was purified to apparent homogeneity. Using E. coli Ung, we observed that Ugi inactivated the enzyme in a noncompetitive manner. Kinetic studies revealed a Ki value (0.14 microM) of approx. twelve-fold lower than Km value (1.7 microM) of glycosylase. Ugi did not act synergistically with free uracil to inhibit E. coli Ung suggesting that uracil and Ugi could share a similar mode of inhibition.

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